

Histopathological and Laboratory Assessment of Visual Dysfunction

by Ralph Heywood*

The currently available methods of assessing ocular toxicity are discussed. Manifestations of ocular toxicity are best described clinically; histopathological examination of the eye is beset with problems of preparing the eye for morphological examination. Electron microscopy is essential to look for chemically induced side effects at the cellular level. Mechanisms of ocular toxicity are poorly understood, and the limitation of animal studies in predicting side effects in man must be appreciated.

The concept of chemical damage to the eye, with effects on vision, is one of the sensitive areas of current toxicology. Because of the problems of adverse reactions it is now required that new chemicals are tested for safety before their general release. Animal studies are essentially comparative, in that spontaneous and induced pathological manifestations are compared in control and treated animals, respectively. The purpose is to identify variations from normal and to define underlying disturbances of physiological mechanisms. The conventional procedure for toxicity studies is to have a control group and three groups of animals given different dose levels of a compound, in order to identify target organ systems and to define no observable effect levels. The regulatory authorities stipulate that these studies must be carried out in a rodent and a nonrodent species. The purpose of this paper is to outline the methods for assessing ocular damage in the commonly used laboratory species, following systemic administration of chemicals, with particular reference to underlying mechanisms of toxicity, and to draw attention to the limitations both in the use of, and understanding of, these approaches to safety evaluation.

The overwhelming number of reports on ocular toxicity (1-5) gives the impression that the eye is a potential target organ of every chemical ever discovered. The interpretation of these reports must be kept in perspective.

*Huntingdon Research Centre, Huntingdon, Cambridgeshire, PE18 6ES, England.

Examination of the Eye

Ocular toxicity can be monitored by using a variety of techniques: those applicable to animals are listed in Table 1. Because toxicological studies concern groups of animals, the techniques adopted must be simple and applicable to the unanaesthetized animal. Examination of the eye may be by direct or indirect ophthalmoscopy, although indirect ophthalmoscopy is preferred. Slit-lamp biomicroscopy is not recommended in the routine ophthalmoscopic examination. Tear flow can be measured by using commercially available Schirmer tear-test papers. Intra-ocular pressure can be measured either by using the Schiøtz' tonometer or preferably, an applanation tonometer.

Functional examination is limited to the testing of the simple reflexes—the fixating reflex, the

Table 1. Examination for ocular toxicity.

General examination
Examination with the ophthalmoscope
Direct
Indirect
Biomicroscopy
Schirmer's test
Ocular pressure
Functional examination
Electrodiagnosis—ERG
Biochemistry
Pathological examination
Light microscopy
Electron microscopy

corneal reflex, the blink reflex and the pupillary light reflex. The fixating and blink reflexes are dependent on the afferent pathways of the optic nerve, optic tract, lateral geniculate body, optic radiation and visual cortex. The efferent pathway is via the superior colliculus, the third, fourth and sixth cranial nerves, and cervical nerves. Constriction of the pupil is dependent on the optic nerve, optic tract, pretectal region with the efferent pathway, the third parasympathetic division, ciliary ganglion and nerve. The consensual response is dependent on decussations in the optic chiasma.

Physiological measurement of retinal integrity can be made by recording electroretinograms. The electroretinogram represents the summated transient electrical activity evoked in response to a light stimulus, of a large number of retinal cells. It is now generally accepted that the outer layers of the retina give rise to the initial negative deflection, or *a* wave, while the activity of the inner layers of the retina is represented by the subsequent positive deflection, or *b* wave. The later positive potential, or *c* wave, is thought to originate in the pigment epithelium. The pattern of response of the rod and cone systems are known to differ, the cones having a higher stimulus threshold than the rods. Brunette and Lafond (6) have demonstrated, in rhesus monkeys, that when stimuli of high intensity are used, the rod and cone responses are superimposed, and that a pure rod response is obtained only by using low-intensity stimuli after complete dark adaptation. The validity of the results obtained from an electroretinographic examination relies heavily on the minimization of all artifacts and the standardization of the recording technique. Subtle changes in the electroretinograms recorded during, or after, treatment of experimental animals with novel compounds must be interpreted with caution and assessed in conjunction with other clinical and ultrastructural histopathological findings. Without close correlation with behavioural or morphological evidence, changes in the electroretinogram are not necessarily attributable to disturbance of visual function. The value of the electroretinographic examination in predictive chemical safety evaluation lies in the provision of supplementary evidence to confirm or refute ophthalmological assessments based on conventional clinical and pathological data.

Considerable problems are encountered in preparing eyes for morphological examination. Immediately after the animal has been killed, the eyes should be dissected from the orbits by using the technique described by Saunders and Jubb (7) and placed in a fixative. It is essential to use a fixative which will rapidly penetrate the sclera. Zenkers-acetic

imposes critical timing but gives good results for the retina. Bouin's fluid is considered to be better for fixing the lens. The fixation, however, most appropriate for routine toxicological laboratories, is Davidson's fluid. Formalin fixation inevitably causes separation of the retinal layers in the rat. It is difficult to avoid inducing artifacts in the taking and fixing of eyes. After fixation, the calottes are removed and the eye embedded in fibro-wax. It is useful if the eye can be correctly orientated before sectioning: this can sometimes be done by reference to the ocular muscles and/or by marking the cornea before fixation. Sagittal sections are cut at 5 μ m and stained with hematoxylin and eosin. For electron microscopy, the eyes are fixed in sodium cacodylate buffered glutaraldehyde for 5 min. The cornea is then removed and the globe returned to the glutaraldehyde for a further 5 min, after which the iris is removed and the eye allowed to fix for a further 10 min. The lens and vitreous are then removed from the cup, which is allowed to fix for a further 45 min. The retina is subsequently post-fixed in 2% osmium in cacodylate buffer for 1 hr before being dehydrated in alcohol and embedded in epoxy resin.

It is necessary to know the range of normal and spontaneous pathological variations common to the test species. In the dog, rat and primate, many spontaneous abnormalities occur congenitally, or as a result of trauma, infection or developmental and ageing processes.

Manifestations of Ocular Toxicity in Laboratory Animals

The Adnexa

Compounds acting on the adnexa of the eye involve the eyelids or affect lachrymal secretion.

Ptosis of the eyelids, although a common sign in acutely intoxicated rats, is rarely observed following chronic administration of compounds. Edema of the eyelids is occasionally seen, often associated with nonsteroidal anti-inflammatory agent administration. Paracetamol intoxication in the dog induced palpebral edema at the dosage level of 450 mg/kg/day with an associated reduction in the secretory activity of the meibomian glands. The dog has a third eyelid, which is extensive and can cover the whole anterior aspect of the eye; compounds affecting the autonomic system can cause relaxation of this third eyelid.

Conjunctival alterations are most frequently caused by changes in the lachrymal secretion and usually involve the cornea.

The secretion of red tears in the rat, chromodacryorrhea, is mainly associated with stress factors, or an overgrowth of the incisor teeth. Harkness and Ridgway (8) have shown that injections of acetylcholine increased almost instantaneously the flow of a dull red secretion, the red color being caused by porphyrins.

Keratoconjunctivitis sicca has been induced in dogs by phenazopyridine hydrochloride (9) and some sulfonamides (10); the hypotensive agent, clonidine, has recently been found to induce keratoconjunctivitis sicca in the rat (11). All anticholinergic compounds given in high dose levels induce keratoconjunctivitis sicca, particularly in the dog. Secretion of lachrymal fluid is not exclusively parasympathetic in origin: stimulation of the sympathetic system also plays a part, and this accounts for the occasional cases of dry eye, seen in dogs given high doses of beta-adrenergic receptor blocking agents.

These conditions of the adnexa are best diagnosed by clinical examination, and, where appropriate, supported by evidence of reduced tear secretion.

Cornea

For a systemically administered compound to affect the cornea it must be secreted in the tears or pass the blood-aqueous barrier. Epithelial erosions are the simplest form for the damage to take, but with continued insult the superficial lesions rapidly can progress to keratitis of varying severity. Some compounds can reach concentrations within the aqueous humor sufficient to damage the corneal endothelium. If damage does occur, the stromal swelling becomes apparent in the whole cornea and the eye appears white and bulges from the orbit. Corneal opacities can be induced in laboratory animals by a variety of compounds, in the rat invariably by morphine (12) or the narcotic analgesics (13). Lipoidal opacities induced by a nitroaniline have been described in the dog (14).

Exacerbation of traumatic lesions and reactivation of old infections can be induced following the administration of steroids.

Toxicity involving the cornea is best diagnosed by clinical examination supported by histopathological examination of the cornea.

Uveal Tract

A side effect of many chemicals is the disturbance of the refractive power of the eye and the ability of the eye to accommodate. The common response of the pupil is dilation or constriction. Dilation of the pupil is induced by the systemic administration of

anticholinergic compounds and parasympatholytic agents: constriction is by direct cholinergic or parasympathomimetic action. The effects on the pupil can be modified by central effects.

The high vascularity of the uveal tract should make this a potential site for chemical immunologic reactions. However, this has not been the case, and in my experience one compound only, an antiviral agent, caused sensitization of the uveal tract in a subhuman primate species, with resultant uveitis.

Lens

The lens arises from ectoderm. It is a biconvex transparent body, separating the aqueous and vitreous chambers. It has no blood supply and relies on the aqueous and, to a lesser extent, the vitreous, for its supply of nutrients. The lens continues to grow through life by layering new fibers over the old fibers, so that the old cells become compressed into the center. The lens undergoes a progression of age-related changes and may also develop opacities. The characteristic response to injury is degeneration.

For a compound to be cataractogenic it must traverse the blood-aqueous barrier to enter the anterior chamber. Toxic levels can be achieved only by high daily dosage, slow excretion or prolonged administration. The selective entrance of drugs into the aqueous is dependent on molecular size and lipid solubility. Large molecules are held back by the capillary membrane. Lipid-soluble substances rapidly traverse the blood-aqueous barrier and reach equilibrium.

Chemically induced lenticular lesions are cataracts and can be classified into two major groups: transient lens opacities and permanent cataracts.

Transient Lens Opacities. Opacities of a transient nature, in which the lens returns to normal within a few hours, have been produced in young rats following the administration of substituted phenothiazines, catecholamines and morphine-like analgesics. Transient lens opacities have been produced in young Beagle dogs following the administration of some tranquilizers, some diuretics and diisophenol. These opacities are usually associated with the anterior capsule, where they appear as crescents around the periphery of the lens, or as discrete opacities on the anterior surface of the lens (Fig. 1). These acute reversible lens opacities are ascribed primarily to changes in the osmolality of the aqueous humor, although other factors may be involved.

Permanent Cataracts. The clinical development of permanent chemically induced cataracts follows distinct courses. There is always a latent period between the administration of a compound and the



FIGURE 1. Transient lens opacity.



FIGURE 2. Opacification of superficial lens fibers.

onset of lenticular change, and this can vary from a few days to several months. Many cataracts start at the equator of the lens. The first sign is the appearance of vacuoles and striation around the equator of the lens. The lens at the equator becomes opaque and appears to have a surrounding halo. Opacification of the superficial lens fibers continues and extends under the anterior or, more frequently, the posterior capsule of the lens. Initially, only those lens fibers immediately below the capsule are involved (Fig. 2). The most common form of chemically induced cataract is that which starts at the posterior pole of the lens. In the dog, these cataracts appear roughly triangular at the posterior pole, involving only subcapsular fibers (Fig. 3). In the rat, chemically induced cataracts show a variety of morphological patterns, but often they first show as triangular opacities at the posterior pole. The most likely explanation of the greater involvement of the posterior cortex of the lens in toxic cataracts is because posteriorly the capsule is thinner and epithelial cells are absent, allowing the toxic agent to penetrate into the superficial fibers more readily. At the posterior pole the lens is at its greatest stress with respect to nutrients and oxygen. The only other manifestation of lenticular toxicity that has been shown is that of a changes in refraction. Changes in the transparency as well as the refraction of the lens nucleus have been recorded following the administration of DMSO (15) and with *p*-chlorophenylalanine (16).

Cataracts are best diagnosed by clinical examination; processing of the lens for histopathological examination is of very limited value. Chemicals with widely differing structures and pharmacological activity have been reported to cause cataracts in laboratory animals. These agents have been reviewed by Gehring (17). Such compounds defy easy class-



FIGURE 3. Triangular posterior polar subcapsular opacity.

ification because the mechanisms of their cataractogenicity are poorly understood.

Cataracts can be induced by feeding high levels of galactose and xylose to rats. Animals made diabetic by the administration of alloxan or streptozotocin or those with high dose levels of progestational agents develop cataracts. Here the common mechanism is the formation of sugar alcohols which accumulate; because they are not easily metabolized they cause hypertonicity and osmotic swelling. Those alkylating agents that are cytotoxic induce cataracts by interfering with cell proliferation. Triparanol, a drug once used in the treatment of hypercholesterolaemia, induces cataracts in the rat and the dog. The opacities appear as peripheral striate opacities, and sudanophilic material has been identified within the lens fibers. It is possible that this type of cataract is a manifestation of phospholipidosis. The cataracts induced by other compounds, such as the sulfonylurea drugs, the chelating agent desferrioxamine, sulfonamides, the steroids such as

methallibure and clomiphene citrate, and chemicals such as diquat, heptachlor and mirex, are of unknown mechanisms. Many of the agents inducing cataracts may do so by specific interference with enzyme systems. The toxic effects of various substances on the lens are quantitatively very different in different species.

Retina

The response of the ocular fundus to chemical insult can be classified into three main categories: hemorrhage; the presence of exudates; and degenerative changes.

Compounds which cause hemorrhage do so by acting directly or indirectly on the clotting mechanism. The blood frequently penetrates the hyaloid membrane and appears in the vitreous as blobs or clots.

Exudates are caused by the escape of plasma

and/or white blood cells from defective blood vessels. Exudates between the pigment epithelium layer and the rod and cone layer can cause partial detachment of the retina (Fig. 4). The quinines, such as imidazoquinazoline and quinine sulfate, can induce this type of change in the dog (18). Occasionally, the compound or a metabolite of the compound can be found to leak from retinal vessels.

The drug-induced retinopathies have been reviewed by Meier-Ruge (19), with the main discussion directed towards the antimalarial chloroquine, and the psychotropic phenothiazine derivatives. Of the three categories of phenothiazines, only the piperidines induce retinal lesions. Of these compounds, thioridazine has been most extensively studied. Retinal lesions can be readily induced with these compounds in the cat and dog; on examination, the retina of the tapetum has a coarse spotted appearance (Fig. 5). The lesions first occur in the primary visual cells, with secondary changes in the pigment epithelium.

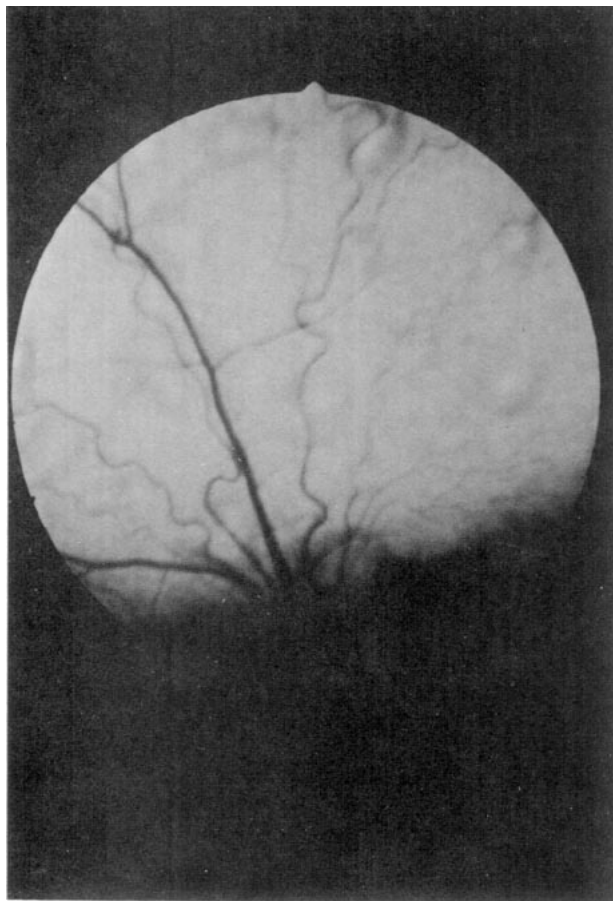


FIGURE 4. Retinal detachment: dog.

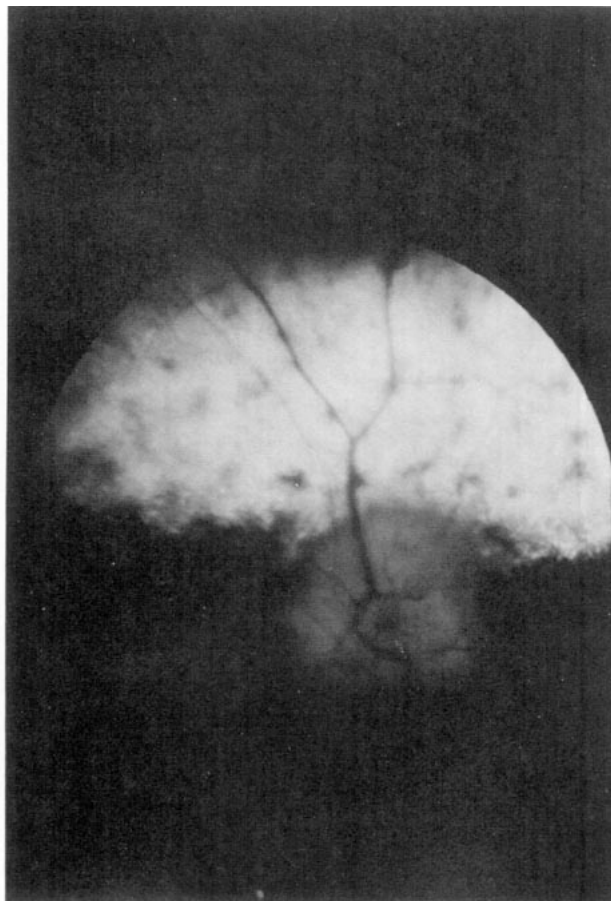


FIGURE 5. Thioridazine toxicity: dog.

Meier-Ruge (20) has shown by histochemical techniques that there is a massive loss of enzyme activity in the ellipsoids of the rods. He is also of the opinion that the absorbent binding to melanin is of importance.

Experimental chloroquine retinopathy has caused considerable controversy over the mechanisms of the toxicity. It is certain that chloroquine binds to the pigmented tissues of the eye (19, 21-23), but it is doubtful whether the pigment binding is of relevance to the primary retinopathy. From work carried out in the albino rat and pigmented strains, rabbits, beagle dogs, cats and the rhesus monkey,

the initial reaction of the retina is the formation of membranous cytoplasmic bodies (myelinoid bodies), and these myelinoid bodies can be induced within one week of starting treatment (Fig. 6). Rosenthal et al. (23) clearly demonstrated that degenerative changes in the eye of the monkey occur in the nucleus and cell body of both the rods and cones and that the pigment epithelial damage was a later manifestation of chloroquine toxicity. In this experiment in the rhesus monkey, very extensive damage occurred in the retina, although there was no clinical or functional evidence of change.

The binding capacity of the pigment tissues of the

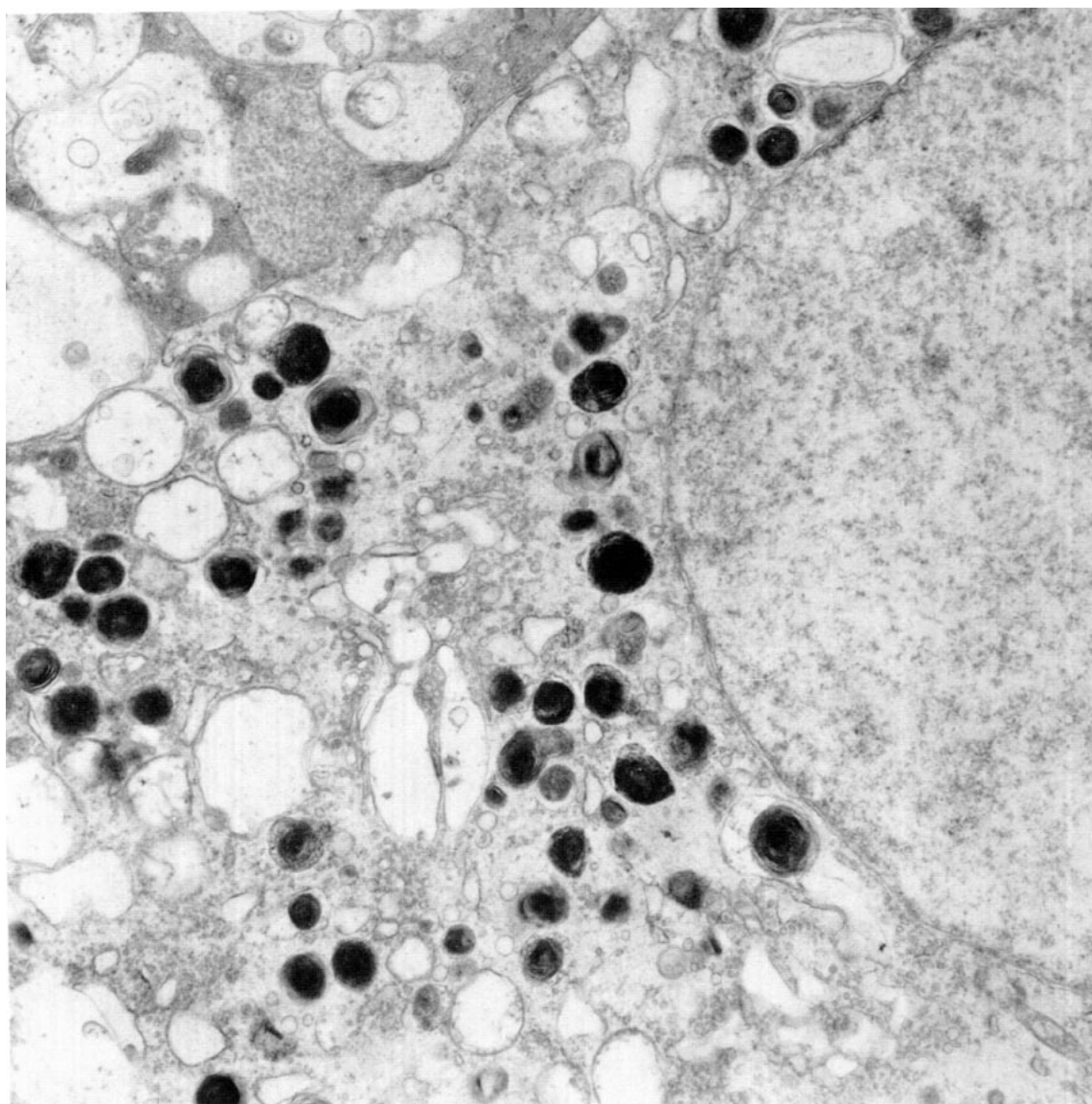


FIGURE 6. Myelinoid bodies. Ganglion cells of rat retina following 5 doses of 100 mg/kg/day of chloroquine. $\times 14,300$.

eye have been demonstrated for many compounds besides chloroquine and the phenothiazines. The beta-blockers, rifampicin, many antiprotozoals, tetracyclines, glycosides and most polycyclic compounds bind to melanin. Compound uptake apparently increases with time, and once bound to melanin it can be retained for long periods. With the majority of compounds there is no resultant retinal toxicity, presumably because the compound is bound in an inactive form. In some cases, as with some phenothiazines and chloroquine, once the binding capacity has been exceeded, direct damage may result.

There are few reports of drug-induced retinal toxicity in the rat. Cyproximide, a psychotropic agent (24), a propionic acid derivative (25) and a

nitrosourea (26) have been recorded as inducing retinopathies in the rat.

The administration of amphophilic compounds to the rat results in the formation of myelinoid bodies in many cell types. These myelinoid bodies are uni- or multicentric. They are particles limited by a single membrane and containing osmiophilic membranes in concentric arrangement.

The formation of myelinoid bodies is a manifestation of chemically induced lysosomal storage disease. Generalized phospholipidosis has been induced by a variety of chemical agents which include antihistamines, hypolipidemics, anti-inflammatories, antidepressants, anorectics and coronary vasodilators (27, 28). In chemical safety evaluation studies it has

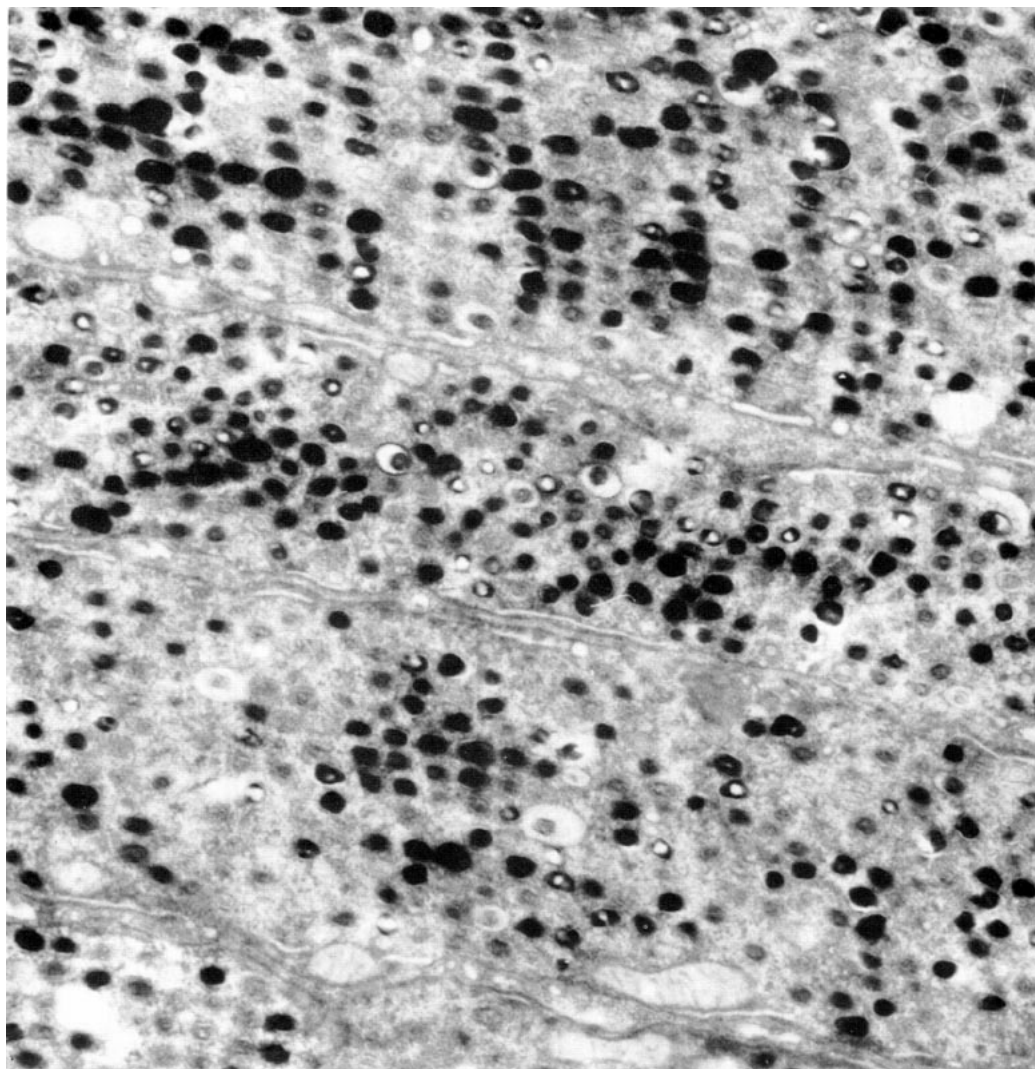


FIGURE 7. Normal tapetal cells: dog. $\times 20,200$.

been the drug-induced pulmonary lipidosis with the accumulation of foamy macrophages which has promoted interest. Drenckhahn and Lullmann-Rauch (29) have focused attention on drug-induced retinal lipidosis, which has been caused by several compounds. Myelinoid bodies have been found in the inner retinal cell types and the pigment epithelium. Different compounds have given different distributions: some compounds, like triparanol, have shown a predilection for pigment epithelial cells, while chloroquine affects mainly the ganglion cells. Some compounds which have been shown to produce a marked generalized phospholipidosis involving lungs, liver and endocrine tissues, peripheral and central nerve cells, and epididymal tissue, have induced only a mild degree of lipidosis in the retina. It

seems that the accumulation of myelinoid bodies within the cell does not lead to cell death, and there is some evidence that, even with the continued administration of compounds, the cells recover from the initial shock, and the accumulated myelinoid is not progressive. Myelinoid bodies tend to disappear after cessation of treatment. The use of electron microscopy is essential to look for these myelinoid bodies. Myelin figures can be produced in tissues fixed in glutaraldehyde, if fixation is prolonged. These figures could be mistaken for pathological change.

Some chemicals cause a loss of tapetal color. The change first appears as patchy fading of the tapetal color, until eventually the entire tapetum has a bleached appearance. No histological change can be

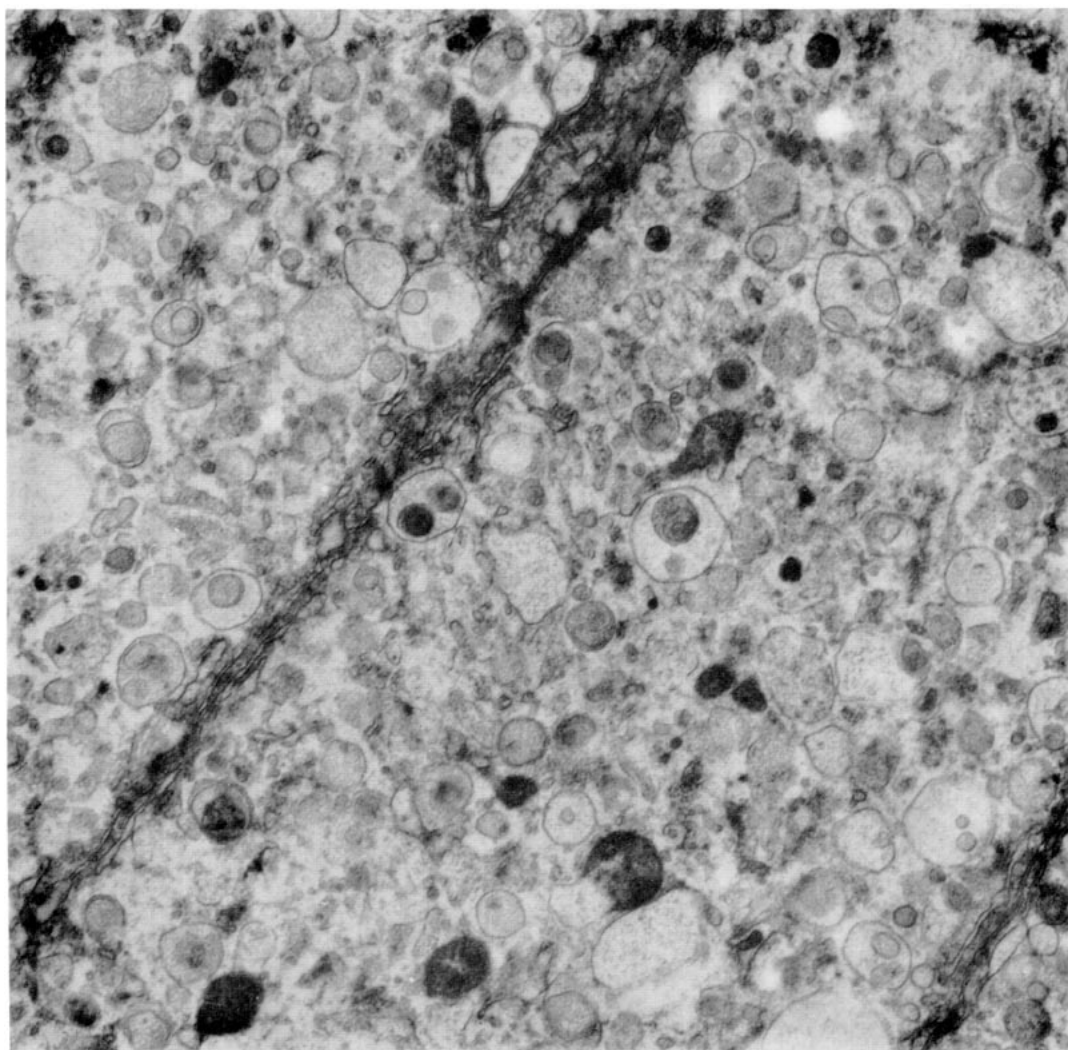


FIGURE 8. Tapetal cells for animals with a bleached tapeta. $\times 20,200$.

detected by light microscopy, but electron microscopy shows the presence of ultrastructural changes. Tapetal cells from normal dogs are characterized by the presence of parallel groups of uniformly dense intracytoplasmic rods (Fig. 7); in animals with bleached tapeta the parallel arrangement of the rods is disrupted, the rods are surrounded by vacuoles which are not uniformly dense, appearing swollen with indistinct outlines (Fig. 8). No functional alteration can be detected by ERG other than slight prolongation of the implicit time of the wave forms evoked in response to low-intensity stimuli in the dark-adapted state. These changes are attributed to the chelating action of these compounds; ethambutol is one such compound (30).

Clinical and functional examinations of the retina are shown to have considerable limitations in detecting toxicity. Histological examination of thick sections will detect only the most gross lesion. The use of electron microscopy is essential to look for chemically induced side effects at the cellular level.

Optic Nerve

The response of the optic nerve is limited: it may show atrophy or it may increase in size. The cases of atrophy show as a reduction of myelinated axons or demyelination of the optic nerve. In the dog, an organophosphate pesticide—ethylthiometon (31)—and clioquinol (32) induce this type of change. Ethambutol has caused demyelination of the optic nerve, chiasm and tract in the monkey (33) and the rat (34).

Edema of the disc is reported in monkeys with methyl alcohol poisoning (35) and trimethyltin acetate (36). Salicylanilide (37) causes vacuolation of the cerebral white matter in the dog and edema of the disc (Fig. 9), and hexachlorophene (38) induces peripapillary exudations in the dog.

Changes in the optic nerve are readily diagnosed by the standard clinical and histopathological methods, although impairment of the pupillary light reflex is frequently the first evidence of damage.

Discussion

The validity of safety evaluations is based on the assumption that extrapolation from animal to man is possible. It is appropriate to ask how predictive are the common laboratory animal species with respect to ocular toxicity.

In a recent survey, in which the toxicological profile of 50 compounds were compared in rodent and nonrodent species, Keywood (39) found that in the dog, 26% of the compounds and, in the monkey species, 7% of the compounds, induced some form

of ocular toxicity, whereas in the rodent, none of the compounds affected the eye. A review of the literature shows some correlations for ocular toxicity between the rodent and nonrodent species—for example, triparanol, some sulfonamides, chloroquine, iminodipropionitrile—but such correlations are rare.

If we try to compare adverse eye reactions in man with toxic findings in animals, again the correlations are poor. The classical cataractogenic agent cited for man is dinitrophenol. Animal models to investigate the cataractogenicity of this drug have been the duck or chicken, special strains of mice, scorbutic guinea pigs, rabbits under 1 month, or 3-week old dogs. It is apparent that current safety evaluation studies in rats and beagle dogs or non-human primates would not have predicted the cataractogenic potential of this compound to man. Cortical posterior subcapsular cataracts have been induced in man, both in children and adults, by a variety of glucocorticoids. Animal experiments have failed to induce changes in the lens. The keratoconjunctivitis sicca described by Wright (40) as part of



FIGURE 9. Edema of the disc: dog.

the mucocutaneous syndrome induced by practolol, was not predicted by conventional animal experimentation. Attempts to reproduce subacute myeloptic neuropathy (SMON) in experimental animals has yielded unreliable and sometimes equivocal results. Worden and Heywood (41) drew attention to the fact that the lesion induced in the dog did not accord with the criteria for SMON in man.

Some findings in animals, have not been confirmed by human clinical experience. In aging monkeys, both controls and particularly animals undergoing long-term administration with progestogens, hyperfluorescent spots around the macula have been described. Fine and Kwapien (42) have shown that these lesions consist almost exclusively of lipid degeneration of the pigment epithelial cells. The significance of this finding in relation to women using oral contraceptives is not known; however, there is no clinical evidence to establish an adverse effect of oral contraceptives on the retina. From the data presented it would appear that all amphophilic cationic compounds have the potential to affect the retina in the rat, and form myelinoid bodies. The same mechanism is evidently not operant in man.

Many compounds affect physiological processes which may involve the visual system but which cannot be predicted from animal experimentation. Of 56 compounds investigated by Laroche and Laroche (43), 34 were found to decrease color discrimination significantly; 18 of these 56 compounds were antibiotics, and nine caused some impairment of vision. Of patients on digitalis, 10-25% experience some visual symptom of toxicity (4). The halucinogens have variable effects on vision (44).

The eye may show side effects from a wide variety of drugs used in clinical medicine. Some of these side effects, although undesirable, must be accepted as unavoidable. Many of the ocular side effects are caused by overdosage or indirect consequences of the drugs' primary action, or by an individual idiosyncrasy.

Target-organ toxicity, induced by high dose levels of test compound in laboratory animals, is of little importance in the assessment of safety of compounds. Safety evaluation should be based on the no observable effect level. As far as the eye is concerned, animal experiments have been shown to have little predictive value for man.

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